TNFα-mediated autophagy inhibition disrupts platelet metabolism and hemostatic function in inflammatory diseases

G. Rojas-Sanchez[1], J. Calzada-Martinez[1], B. McMahon[2], O. Esparza[3], G. Hernandez[3], D. Le[3], E. P. Wartchow[4], K.Jones[5], L. Ting[6], C. Jankowski[7], M. R. Kelher[8], M. Manco-Johnson[9], T. Nemkov[10], A. D'Alessandro[10], A. Thorburn[11], P. Maycotte[12], J.A. López[1,13] and P. Davizon-Castillo[1,1]

[1]Bloodworks Research Institute; [2]University of Colorado, School of Medicine Hematology; [3]Department of Pediatrics Hematology/Oncology and Bone Marrow Transplantation, University of Colorado; [4]Electron Microscopy Laboratory, Children's Hospital Colorado; [5]Bioinformatics Solutions; [6]Stasys Medical; [7]University of Colorado College of Nursing; [8]Univeristy of Colorado School of Medicine, Department of Surgery; [9]Department of Pediatrics, Hemophilia and Thrombosis Center, University of Colorado; [10]Biochemistry and Molecular Genetics Department, University of Colorado; [11]Department of Pharmacology, University of Colorado; [12]Centro de Investigación Biomédica de Oriente, Instituto Mexicano del Seguro Social,; [13]Division of Hematology and Oncology, University of Washington; [14]Department of Pediatrics, Hematology/Oncology, University of Washington.

Bloodworks Northwest

Earl W. Davie Symposium 2024

14

Introduction

Platelets are critical for hemostasis, undergoing structural and functional changes upon activation that require substantial mitochondrial ATP production. Mitochondrial dysfunction is linked to inflammatory conditions with high rates of thrombotic and hemorrhagic complications, including cardiovascular disease (CVD), cancer, diabetes, myeloproliferative neoplasms (MPNs), and rheumatoid arthritis (RA), as well as aging. We demonstrated that exposure to TNFα increases mitochondrial mass and alters the expression of genes involved in autophagy in platelets[1].

Autophagy removes damaged or long-lived proteins, lipids, carbohydrates, and organelles. Mitophagy, a selective form of autophagy, degrades dysfunctional mitochondria, serving as a quality control mechanism to maintain mitochondrial integrity and cellular bioenergetics. Defective mitophagy leads to the accumulation of dysfunctional mitochondria, and decreased ATP levels[2].

Altered platelet autophagy has been recently observed in patients with inflammatory conditions such as sepsis, diabetes, and ischemia/reperfusion[3-5]. However, the mechanisms underlying these alterations and their implications for hemostasis remain unclear.

Hypothesis

TNFα inhibits autophagy and mitophagy in platelets, disrupting their metabolism and hemostatic function.

Results

MPN Platelets Contain Dysfunctional Mitochondria, Are Hypometabolic, and Exhibit an Aberrant Functional Profile

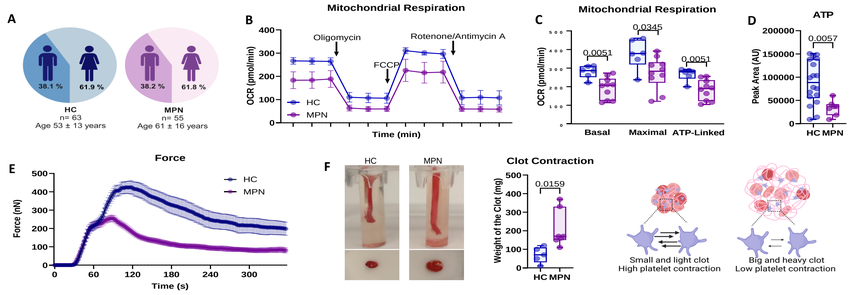


Figure 1. ***MPN Platelets Contain Dysfunctional Mitochondria, Are Hypometabolic, and Exhibit an Aberrant Functional Profile.*** **(A)** Demographic information from healthy controls (HC) and patients with JAK2^{V617F+} polycythemia vera (hereafter referred to as MPN). **(B)** Mitochondrial respiration. **(C)** Basal, maximal, and ATP-linked respiration. **(D)** ATP levels. **(E)** Platelet force assay with the ATLAS system. **(F)** Clot contraction using normalized platelet counts.

Autophagy and Mitophagy are Blocked in MPN Platelets

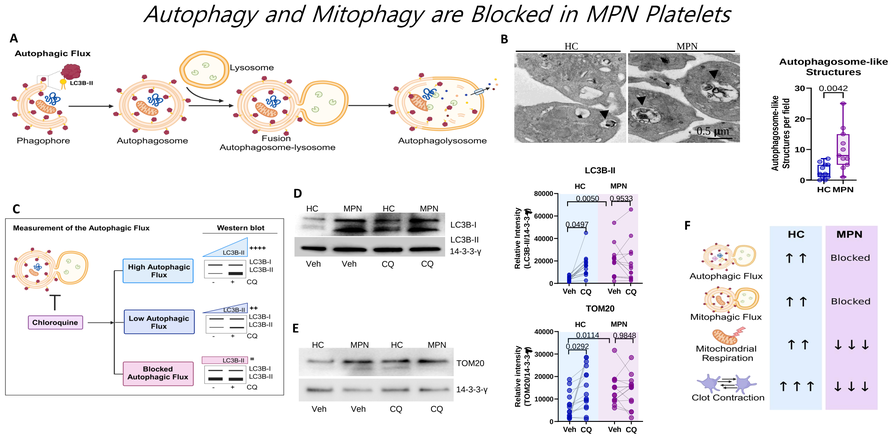


Figure 2. Autophagy and Mitophagy are Blocked in MPN Platelets. **(A)** Diagram of the autophagic flux. **(B)** Transmission electron microscopy analysis. **(C)** Methodology for determining the autophagic flux status. **(D)** Immunoblot of LC3B-II protein. **(E)** Immunoblot of TOM20 protein. **(F)** Summary..

Results

Autophagy Blockade Decreases Mitochondrial Respiration and Platelet Contraction

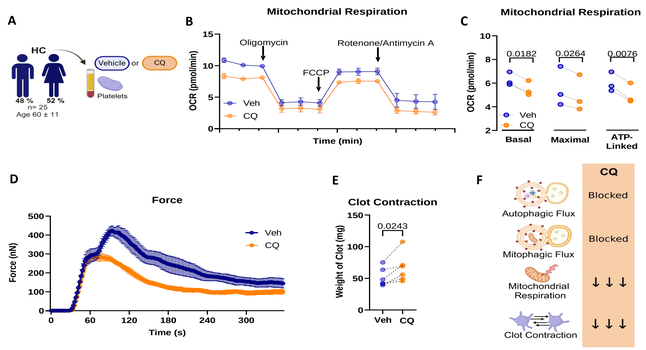


Figure 3. ***Autophagy Blockade Decreases Mitochondrial Respiration and Platelet Contraction.*** **(A)** Methodology. **(B)** Mitochondrial respiration. **(C)** Basal, maximal, and ATP linked respiration. **(D)** Platelet force assay. **(E)** Clot contraction. **(F)** Summary.

STX17 Modulates Platelet Metabolism and Function

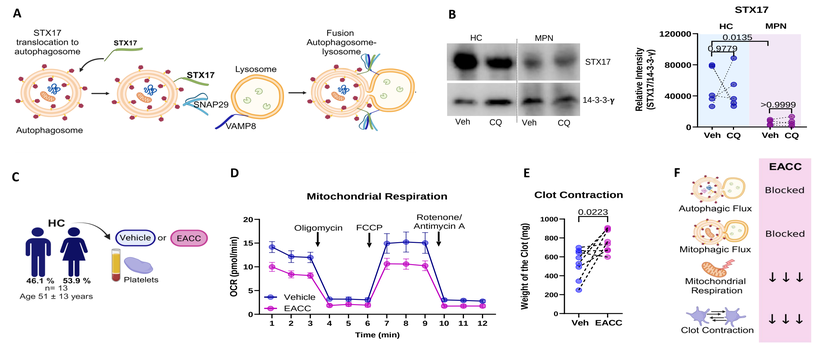


Figure 4. STX17 Modulates Platelet Metabolism and Function. (A) Diagram of STX17 mechanism in the autophagic process. **(B)** Immunoblot of STX17. **(C)** Methodology. **(D)** Mitochondrial respiration. **(E)** Clot contraction. **(F)** Summary.

Platelets from a Mouse Model of RA/IBD Have a Blocked Autophagy and Mitophagy

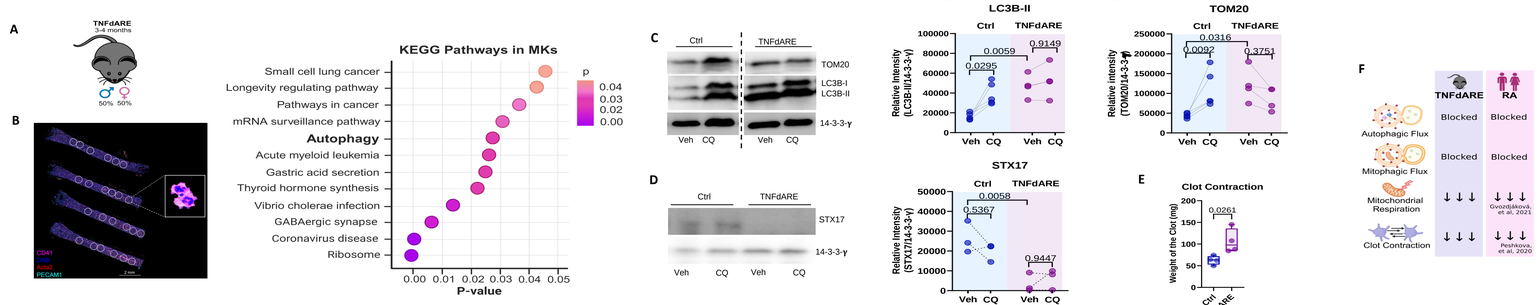


Figure 5. Platelets from a Mouse Model of RA/IBD Have Blocked Autophagy and Mitophagy. STX17 Modulates Platelet Metabolism and Function. (A) TNFdARE mice is a RA/IBD model. **(B)** Spatial RNA transcriptomics from megakaryocytes from the bone marrow. **(C)** Immunoblot of LC3B-II and TOM20. **(D)** Immunoblot of STX17. **(E)** Clot contraction. **(F)** Summary.

Anti-TNFα Therapy Restores STX17, Autophagy, and Mitophagy in Platelets from a Mouse Model of Aseptic Inflammation

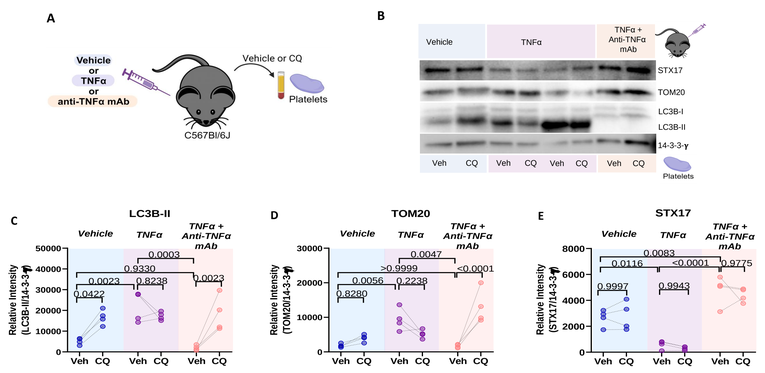
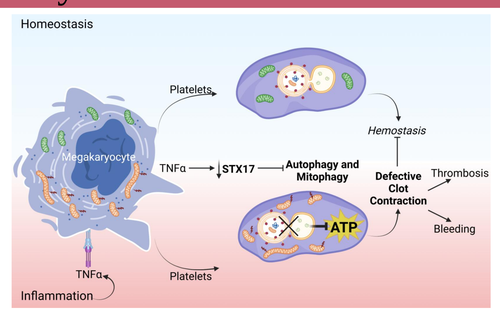


Figure 6. Anti-TNFα Therapy Restores STX17, Autophagy and Mitophagy in Platelets from a Mouse Model of Aseptic Inflammation. **(A)** Methodology. **(B)** Immunoblot of LC3B-II, TOM20, and STX17. Analysis of **(C)** LC3B-II, **(D)** TOM20, **(E)** STX17.

Acknowledgments

This work was supported by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health under the award number K99HL156058-01, the Hemophilia and Thrombosis Center from the University of Colorado School of Medicine, and the ASH-AMFDP.

Summary



References

1. Davizon-Castillo, P., McMahon, B., Aguila, S., Bark, D., Ashworth, K., Allawzi, A., Campbell, R.A., Montenont, E., Nemkov, T., D'Alessandro, A., et al. (2019). TNF-α-driven inflammation and mitochondrial dysfunction define the platelet hyperreactivity of aging. Blood *134*, 727-740. 10.1182/blood.2019000200.
2. Wang, S., Long, H., Hou, L., Feng, B., Ma, Z., Wu, Y., Zeng, Y., Cai, J., Zhang, D.-w., and Zhao, G. (2023). The mitophagy pathway and its implications in human diseases. Signal Transduction and Targeted Therapy *8*, 304. 10.1038/s41392-023-01503-7.
3. 1. Schwertz, H., Rowley, J.W., Portier, I., Middleton, E.A., Tolley, N.D., Campbell, R.A., Eustes, A.S., Chen, K., and Rondina, M.T. (2022). Human platelets display dysregulated sepsis-associated autophagy, induced by altered LC3 protein-protein interaction of the Vici-protein EPG5. Autophagy *18*, 1534-1550. 10.1080/15548627.2021.1990669.
4. 1. Lee, S.H., Du, J., Stitham, J., Atteya, G., Lee, S., Xiang, Y., Wang, D., Jin, Y., Leslie, K.L., Spollett, G., et al. (2016). Inducing mitophagy in diabetic platelets protects against severe oxidative stress. EMBO Molecular Medicine *8*, 779-795. 10.15252/emmm.201506046.
5. 1. Zhang, W., Ren, H., Xu, C., Zhu, C., Wu, H., Liu, D., Wang, J., Liu, L., Li, W., Ma, Q., et al. (2016). Hypoxic mitophagy regulates mitochondrial quality and platelet activation and determines severity of I/R heart injury. eLife *5*, e21407. 10.7554/eLife.21407.